



**NTP**

National Toxicology Program

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# Seizures in NTP F344/N Rats

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# Overview

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## Introduction to Seizures in NTP F344/N rats

- Background information
- Studies involved
- Nature, incidence, frequency and time of onset of seizures

## General

- Etiology of seizures in rodents
- Histopathological changes resulting from seizures

## Investigation

- Possible Etiology
- Potential impact on studies

## Conclusions

# Background Information

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- Since 2000, seizures have been observed in treated, control, and sentinel male and female F344/N rats in NTP carcinogenicity studies
- Rats derived from a single lineage
- Individually housed rats (inhalation and dermal studies)
- Studies were performed at 3 laboratories
- Similar time of onset of seizures across the studies
  - Range: 40-79 weeks in females, 32-104 weeks in males

# Studies Involved

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## 5 Inhalation Studies

- $\alpha$ -Methylstyrene (AMS)
- **Divinylbenzene-HP (DVB)**
- **Methyl isobutyl ketone (MIBK)**
- Cumene
- Propargyl alcohol

# Studies Involved

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## 2 Dermal Studies

- **Diisopropylcarbodiimide (DIC)**
- Bis(2-Chloroethoxy) methane

# Nature of Seizures

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- Variable duration, majority short-lived: <60 seconds
- Variable intensity: minimal to mild
- Abnormal hunched posture and chewing movements
- Occasional clonic spasms of the limbs
- Uncommonly: more pronounced jerking movements
- Short post-ictal recovery period: 30-120 seconds

## Nature of Seizures (cont.)

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- Noted during daily routine handling/animal care activities (predominantly in the morning)
- Handling is not a prerequisite for the onset of seizures

# Incidence and Multiplicity of Seizures

	$\alpha$ -Methylstyrene	Cumene	Divinylbenzene -HP	Methyl Isobutyl Ketone	Propargyl Alcohol
<b>Male</b>	<b>9/200</b>	<b>6/200</b>	<b>8/200</b>	<b>14/200</b>	<b>12/200</b>
Control	0	0	1 1	2 1,1	1 6
Low	3 1,3,5	1 2	4 1,2,7,8	3 2,2,4	5 1,2,3,3,5
Middle	1 1	4 1,1,1,3	1 3	4 1,1,3,3	4 1,2,3,5
High	5 1,2,3,6,10	1 1	2 1,2	5 1,2,3,5,17	2 1,2
Trend P value	0.016	0.242	0.500	0.112	0.500
<b>Female</b>	<b>16/200</b>	<b>24/200</b>	<b>14/200</b>	<b>36/200</b>	<b>21/200</b>
Control	2 1,3	6 1,2,2,3,4,4	1 2	12 1,1,1,2,3,3,4,5,6,7,10,10	3 1,1,3
Low	2 1,2	8 1,1,1,1,1,1,3,1	2 3,5	4 2,4,4,6	3 1,3,4
Middle	3 1,7,9	5 1,1,2,4,5	6 2,2,2,2,4,5	6 1,2,3,3,3,4	6 1,1,2,3,5,11
High	9 1,1,2,3,3,3,3,4,8	5 2,3,4,10,11	5 1,1,1,2,2	14 1,1,1,1,2,4,5,6,6,6,7,16,25,27	9 1,1,2,2,3,4,5,5,13
Trend P value	0.001	0.278	0.038	0.131	0.013

Number of animals with seizures    Frequency of seizures per animal

# Incidence and Multiplicity of Seizures

	$\alpha$ -Methylstyrene	Cumene	Divinylbenzene -HP	Methyl Isobutyl Ketone	Propargyl Alcohol
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<b>Control</b>	<b>0</b>	<b>0</b>	<b>1</b> 1	<b>2</b> 1,1	<b>1</b> 6
<b>Low</b>	<b>3</b> 1,3,5	<b>1</b> 2	<b>4</b> 1,2,7,8	<b>3</b> 2,2,4	<b>5</b> 1,2,3,3,5
<b>Middle</b>	<b>1</b> 1	<b>4</b> 1,1,1,3	<b>1</b> 3	<b>4</b> 1,1,3,3	<b>4</b> 1,2,3,5
<b>High</b>	<b>5</b> 1,2,3,6,10	<b>1</b> 1	<b>2</b> 1,2	<b>5</b> 1,2,3,5,17	<b>2</b> 1,2
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<b>P value</b>	<b>0.016</b>	<b>0.242</b>	<b>0.500</b>	<b>0.112</b>	<b>0.500</b>
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<b>Low</b>	<b>2</b> 1,2	<b>8</b> 1,1,1,1,1,1,3,1	<b>2</b> 3,5	<b>4</b> 2,4,4,6	<b>3</b> 1,3,4
<b>Middle</b>	<b>3</b> 1,7,9	<b>5</b> 1,1,2,4,5	<b>6</b> 2,2,2,2,4,5	<b>6</b> 1,2,3,3,3,4	<b>6</b> 1,1,2,3,5,11
<b>High</b>	<b>9</b> 1,1,2,3,3,3,3,4,8	<b>5</b> 2,3,4,10,11	<b>5</b> 1,1,1,2,2	<b>14</b> 1,1,1,1,2,4,5,6,6,6,7,16,25,27	<b>9</b> 1,1,2,2,3,4,5,5,13
<b>Trend</b>					
<b>P value</b>	<b>0.001</b>	<b>0.278</b>	<b>0.038</b>	<b>0.131</b>	<b>0.013</b>

Number of animals with seizures    Frequency of seizures per animal

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Number of animals with seizures    Frequency of seizures per animal

# Incidence of seizures across studies

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## Incidence of Seizures in Females

Control: 9.6% (2.0 - 24.0%); n = 250

Treated: 11.6% (8.7 - 16.0%); n = 750

## Incidence of Seizures in Males

Control: 1.6% (0.0 - 4.0%); n = 250

Treated: 6.0% (4.0 - 8.0%); n = 750

# Week of Onset of Seizures

Dose Group	$\alpha$ -Methyl styrene	Cumene	Divinylbenzene	Methyl Isobutyl Ketone	Propargyl Alcohol
Male					
Control	-	-	100	95	76
Low	69	97	56	67	42
Middle	104	32	74	57	55
High	70	103	56	65	57
sentinel	-	77	-	-	75
Female					
Control	62	58	76	63	51
Low	49	50	79	68	45
Middle	50	48	41	50	51
High	49	43	59	40	45
sentinel	-	-	-	-	41

# Summary

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- Seen in treated, control and sentinel animals
- Seizure prone animals tended to have multiple episodes
- Number of seizures/animal over the course of study was generally low - majority having  $\leq 5$ /animal
- Females were apparently more susceptible than males
  - higher incidence and /or multiplicity of seizures

# Summary

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- Treatment appeared to exacerbate the incidence and/or multiplicity of seizures in some studies
- Treatment appeared to shorten the time of onset in males and/or females

# Etiology of Seizures

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- Major metabolic derangement
  - Often no associated histopathology
- Idiopathic
  - underlying CNS disorder cannot be identified
  - In these cases, a genetic basis is generally suspected
  - EL (epilepsy) mice - genetic model of idiopathic epilepsy in man
  - animals reported to experience 25-30 seizures during routine weekly cage changing
  - Recurrent seizures were associated with increased glial cells
  - with or without neuronal loss

# Etiology of Seizures

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- Exposure to neurotoxicants
  - Generally results in recognizable morphological changes, which may be irreversible
  - Toxic agents tend to produce primary lesions in one specific target structure eg blood vessels, meninges, neuronal cell bodies or axons
  - However, Identification of abnormal foci resulting in seizures can be extremely difficult to achieve

# Seizure-Induced Pathology

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- Histopathological lesions caused by seizures arise due to the temporary hypoxia/anoxia
- Results in degeneration/necrosis of metabolically active neurons
- Pyramidal neurons of the dorsal hippocampus (particularly CA1 and CA3) are most susceptible to degeneration
- Glial cells typically proliferate around damaged neuronal cell bodies

# Investigation

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- Possible etiology of the seizures
- Potential impact on study interpretation

# Possible Etiology

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- Environmental conditions
  - Housing
  - Noise levels
  - Temperature/humidity
  - Diet
  - Water
- Histopathological basis
  - Routine histopathology
  - Serial sections of brain on subset animals across studies

## Possible Etiology - Environmental Conditions

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- Housing
  - Only seen in studies where rats are individually housed
  - Seizures seen in studies conducted at 3 different laboratories
- Environmental noise
  - Measured at various times, under various conditions
  - Considered insufficient to induce audiogenic response
- Temperature and humidity
  - Within acceptable limits

## Possible Etiology - Environmental Conditions

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- NTP 2000 Diet
  - Routinely evaluated
  - No deviation in composition, contaminants, nutrients
  - Mg levels in NTP 2000 diet - favorable when compared with NIH-07 diet
- Drinking water supply
  - Did not exceed the U.S. Environmental Protection Agency (EPA) maximum contaminant levels.

## Possible Etiology - Histopathological Basis

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- Histopathology
  - Routine histopathology
  - Step section examination of subset of animals

## Possible Etiology - Routine Histopathology

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- Standard 3 sections include fore, mid and hindbrain
- Included major sites expected to see lesions resulting from seizure activity
- No treatment-related neoplastic or non-neoplastic changes in CNS in either sex in any of the inhalation studies

# Possible Etiology - Routine Histopathology

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## Statistical analysis of background lesions and seizures

- To determine - association between seizures and brain or pituitary gland pathology
- Analyses were conducted on each study separately, and data from all 5 studies, combined
- Pituitary Gland
  - adenoma and/or carcinoma, hyperplasia, hemorrhage, cyst
- Brain
  - Primary and metastatic tumors,
  - inflammation, gliosis, necrosis, neuronal degeneration, demyelination
  - Vascular disturbances - hemorrhage, thrombosis, infarct congestion
  - Compression, hydrocephalus

## Result

No statistically significant association between CNS Pathology and seizures

## Possible Etiology - Step Section Examination

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- To maximize the probability of detecting lesions - examined in detail a subset of (12) animals from across the 5 studies
  - Terminally sacrificed animals
  - Females only
  - 4 Controls - no history of seizures
  - 4 Controls - clinical history of seizures
  - 4 Treated females - clinical history of seizures
  - Clinically affected animals with a high number of recorded seizures
- Step sections
  - 30  $\mu\text{m}$  intervals
  - approximately 25 slides per animal

## Possible Etiology - Step Section Examination

Group Examined	(No: animals) Chemical	No: seizures
Control Females No history of Seizures	(2) $\alpha$ -Methylstyrene	0
	(2) Divinylbenzene-HP	0
Control Females With history of Seizures	(1) Cumene	4
	(3) Methyl isobutyl ketone	2,10,10
Treated Females With history of Seizures	(1) $\alpha$ -Methylstyrene	7
	(1) Cumene	10
	(1) Methyl isobutyl ketone	16
	(1) Propargyl Alcohol	11

## Result of Step Section Examination

	Control animals no seizures	Control animals with seizures	Treated animals with seizures
<b>Total number animals examined</b>	<b>4</b>	<b>4</b>	<b>4</b>
<b>Total number of seizures</b>	<b>0</b>	<b>26</b>	<b>43</b>
<b>Number of animals with Axonopathy</b>	<b>4</b>	<b>1</b>	<b>3</b>
<b>Severity grade (per section)</b>	<b>0-2</b>	<b>0-2</b>	<b>0-3</b>

- Axonopathy (axonal degeneration) - only consistent finding
- Characterized by swelling of the axon and fragmentation of the surrounding myelin sheaths when present
- Trigeminal nerve tract: motor and sensory nerve supplying face
- Medial longitudinal Fasciculus: nerve fiber bundle extending between midbrain and spinal cord. Interconnects various cranial nerve cell nuclei

# Result of Step Section Examination

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- No association was found between axonopathy and seizure activity
- No identifiable lesions that may have induced seizures
- No lesions resulting from seizure activity were seen

# Result of Investigation

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- The etiology of seizures in these studies was not determined
- No environmental factors identified which may have resulted in seizures
- No CNS histopathological changes associated with seizures

# Potential Impact on Study Interpretation

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- Seizures were sporadic and infrequent over the course of the 2 year studies
- Although the etiology of seizures was not determined, they occurred in treated, control and sentinel animals and were therefore not considered a direct effect of treatment
- Appears that treatment may have exacerbated incidence, multiplicity and/or accelerated the time of onset of seizures

# Potential Impact on Study Interpretation

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- No effect on body weight gain
- Not associated with the death of any animals
- CNS was not a target organ in any of these studies
- No CNS histopathology resulting from seizures

# Conclusion

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- It was considered unlikely that the occurrence of seizures had a major impact on any of the treatment-related toxicological or carcinogenic effects or on their interpretation.
- Therefore the integrity of these studies was not considered to be compromised



## Result of Step Section Examination

Lesion	$\alpha$ Methylstyrene	Cumene	Divinylbenzene	Methyl isobutyl ketone	Propargyl Alcohol
<b>Total number animals examined</b>	<b>3</b>	<b>2</b>	<b>2</b>	<b>4</b>	<b>1</b>
<b>Number of animals with seizures</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>4</b>	<b>1</b>
<b>Axonopathy</b>	<b>3</b>	<b>0</b>	<b>2</b>	<b>2</b>	<b>1</b>
Trigeminal Tract Cranial nerve V	3	0	2	2	1
Medial Longitudinal Fasciculus	3	0	2	0	1
Other sites	1	0	2	0	0
<b>Severity grade of lesion(s)</b>	<b>1-2</b>	<b>-</b>	<b>1-3</b>	<b>1</b>	<b>1</b>

### Severity Grade

1 = Minimal (total of 1 affected axon/slide)

2 = Mild (total of 2-4 affected axons/slide)

3 = Moderate (total of 5-9 affected axons/slide)

# Result of Step Section Examination

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<b>Severity grade (per section)</b>	<b>0-2</b>	<b>0-2</b>	<b>0-3</b>

## Severity Grade

1 = Minimal (total of 1 affected axon/section)

2 = Mild (total of 2-4 affected axons /section)

3 = Moderate (total of 5-9 affected axons /section)

# Axonopathy

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- Neurons/Nerve cell bodies
  - are located within the grey matter
- Axons
  - carry impulses away from nerve cell body and are
  - predominantly located in the white matter and cranial nerve tracts
- Axonopathy
  - Is the degeneration of an axon
  - Characterized by swelling of the axon and fragmentation of the surrounding myelin sheaths when present.

# Axonopathy

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- Axonopathy
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  - Characterized by swelling of the axon and fragmentation of the surrounding myelin sheaths when present.
- ♦ Trigeminal nerve tract
  - Trigeminal nerve - motor and sensory nerve supplying face
- ♦ Medial longitudinal Fasciculus
  - nerve fiber bundle extending between midbrain and spinal cord
  - Interconnects various cranial nerve cell nuclei